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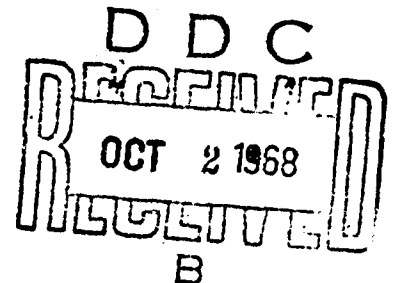
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A CHANGE OF THE PROPERTIES OF THE EV PASTEURELLA PESTIS
STRAIN UNDER THE EFFECT OF ENTEROBACTERIA ANTISERA,
AND THE NUCLEOTIDE COMPOSITION OF ITS DNA

[Following is the translation of an article by R.S. Mikhaylova and M.L. Bekker*, Stavropolskiy Branch of the "Mikrob" Scientific-Research Antiplague Institute, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 10, 1966, pages 25-29. It was submitted on 1 March 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

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In recent years data have been obtained which indicate the presence of antigenic bonds between the plague microbe and certain species of enterobacteria (Sokolova, 1959; Zaplatina, 1960; Shantarenko, 1960; and Martinevskiy, 1963). It has been established that various strains of enteric bacteria are sensitive to plague bacteriophage (Flu, 1927, 1946; Lalazarov, 1948; Adayeva, 1951; Timofeyeva, 1957; Girard, 1957, and others). Smith and Burrows (1962) detected in P. pestis the presence of common receptor zones of bacteriocins with certain representatives of Enterobacteriaceae. Earlier we (Bekker et al., 1962) had established that the nucleotide composition of DNA in the plague microbe is similar to that in enteric bacteria. To a certain degree this testifies to the genetic bonds between the stated microorganisms. It should be noted that Smith and Burrows (1962) also give attention to the relationship of these species of bacteria.

In the process of clearing up the interrelationship of the plague microbe with bacteria of the enteric group we studied the effect of sera against certain species of enterobacteria on the plague causative agent. In the present communication we are presenting the results of cultivating the EV vaccine strain in liquid medium with the appropriate immune sera.

The sera were obtained by means of immunizing rabbits with heated cultures in the variation of Kaufman (1959). In this manner we obtained sera against S. typhimurium No. 256 (titer 1:5120), S. enteritidis No. 527 (titer 1:1280), E. coli 0111 (titer 1:640) and E. coli communis No. 479 (titer 1:5120). The stated strains of enteric bacteria were isolated from rodents and birds (except for E. coli 0111) and possessed a specific antigenic community with the plague microbe.** As a control we used the agglutinating serum to the P. pestis EV strain (titer 1:1280) and normal rabbit serum. The stated sera were added to Hottinger broth of the same

**Trudy of the Armyanskaya Antiplague Station, 1964, No. 3, page 143.

series in a volume of 5-10%. The culture of EV strain was incubated on these media in test tubes at 37° for 2 days and at 28° for 5 more days. After this they were resown on analogous medium. In this manner the initial culture was passaged on each serum 10 times. With each passage a seeding on agar plates was performed. Colonies which differed morphologically were selected for a subsequent detailed study, which was carried out for a period of 2 years.

Apart from the main properties, studied by the generally used methods, in the most changed subcultures we determined the relation of the bases in DNA by the method of paper chromatography (Spirin and Belozerskiy, 1956; Spirin et al., 1957) in a Kirby solution (methanol:HCl:H₂O in a ratio of 70:20:10). For this the selected culture of the EV strain was incubated in separating flasks with Hottinger agar for 2 days at 28°, the bacteria was washed from the medium with physiological solution, rinsed with the same solution and dried with alcohol and ether. The dry mass of bacteria was hydrolyzed with 0.5 N. NaOH at 37° for 18 hours, the DNA was precipitated by acidification with perchloric acid, extracted from the precipitate with 10% NaCl, precipitated with alcohol, dried and hydrolyzed with 72% HClO₄ in a sealed ampoule. The hydrolyzate was placed on washed chromatographic paper and separated in the above mentioned solvent by the descending method for a period of 18-20 hours. The spots of the bases were identified on the ultramicroscope, cut out, and the bases extracted from the paper with 0.1 N.HCl. Spectrophotometry of the extracts and the calculation of the amount of bases (in mole%) were performed by the method described by Spirin and Belozerskiy (1956).***

***In Table 2 we present the average values, obtained as a result of 12 parallel determinations on two hydrolyzates.

In the process of passaging the EV strain in broth with immune sera against enterobacteria we obtained variants which differed quite sharply from the initial culture. Already with the 3-5th passage, together with the unchanged colonies we recorded smooth and rough colonies, of a trabecular structure, with a tapering, slightly raised center without a peripheral zone, chromogenic and achromogenic, dwarfish and gigantic in size, but typical in structure, and also colonies with features, typical for the causative agent of pseudotuberculosis - large, hummocky, with a coarse peripheral zone, concluding format on early. During the course of the passages the phenomena of dissociation increased. They were particularly sharp when the strain was influenced by the antisera of E. coli communis No. 479 and S. typhimurium No. 256.

A study of the cultural-biochemical properties and the investigation with specific sera and bacteriophage showed that some of the subcultures (primarily those obtained from colonies of the "pseudotuberculosis type" and smooth) acquired individual features which were not inherent to the EV vaccine strain and to the plague microbe in general. These were

expressed in the appearance of a fermentation activity in respect to rhamnose, glycerine and urea, in the loss of nitrifying and denitrifying ability, and growth on "hungry" media. Certain variants possessed a resistance to plague bacteriophage and were not agglutinated by anti-plague serum. In a number of cases the changes were so considerable that the resulting variants, based on a combination of differential features, could be related to the causative agent of rodent pseudotuberculosis (Table 1).

Repeated checks, performed after 1½ and 2 years, showed that the new properties, acquired by the EV strain under the influence of sera against enteric bacteria, were preserved, as a rule, quite persistently. Here a distinct tendency was observed for the further mutability of the resulting variants in the direction of their displaying additional features, not inherent to the plague microbe, but characteristic for P. pseudotuberculosis rod. Thus, after prolonged cultivation on artificial nutrient media, certain subcultures, which in the first weeks after passaging were distinguished from the initial EV strain by only one feature (fermentation of rhamnose on the 4-5th day, splitting of urea, etc.), turned out to be very close to the stated causative agent. Thus, these tests clearly demonstrated regularities, characteristic for correlative mutability (Zhukov-Verezhnikov, 1957).

It should be noted that this phenomenon was observed both in the cases where the resulting variants were preserved under refrigerated conditions without reseeded, and also during numerous reseedings.

During the cultivation of the EV strain in broth with homologous serum we recorded mainly the phenomenon of morphological dissociation. In not one of the subcultures studied did we note the appearance of properties, characteristic for the causative agent of rodent pseudotuberculosis.

The culture of the EV strain, passaged on the medium with normal rabbit serum, completely corresponded to the initial one (18 subcultures were studied after the 4-10th passage).

Since certain variants of the EV strain underwent considerable changes, it could have been expected that in these cases we would observe shifts in the ratio of DNA bases, similar to those noted by Spirin and Belozerskiy (1957) during the mutation of bacteria from the enteric group. However, in the determination of the DNA composition in the stated variants it turned out that it did not change and corresponded to that in the initial strain (Table 2).

As is known, mutation of a spontaneous nature was observed in the plague microbe for the first time by Beasonova et al. (1936). Subsequently mutation of such a nature was described as a result of the influence of specific bacteriophage (Tumanskiy, 1937; Korobkova, 1937; Zhukov-Verezhnikov

and Khvorostukhina, 1940, and others), under the influence of antibiotics (Lalazarov, 1964), and under conditions where the plague causative agent is resident in an immune organism (Tumanskiy and Kozlovskaya, 1944). Similar trends in mutability were also observed in our tests under the influence of heterologous sera. Attention is merited by the fact that all the above mentioned factors, different in their nature, caused, all things considered, monotypic changes in the plague microbe. The mutation of the latter took place only in the direction of acquiring the properties of the causative agent of rodent pseudotuberculosis.

In this scheme attention is merited by the fact that the significant shifts in cellular metabolism, observed in our tests, were not accompanied by changes in the nucleotide composition of DNA. This indicated, on the one hand, that the DNA composition is apparently not affected, with the help of which it is possible to detect a similar nature of deviation in the hereditary apparatus of the plague microbe. On the other hand, it is possible that the direction and range of the plague microbe mutability is determined primarily by the homogeneousness of DNA nucleotide composition in causative agents of plague and rodent pseudotuberculosis (Bekker, et al., 1962). It is curious that as a rule those features appear in the plague causative agent which could have been lost by it during the process of evolution.

Further investigations are necessary for explaining the mechanism of mutability in the plague microbe under the influence of heterologous sera. Preliminarily it can only be proposed that in this case there took place a blocking of individual components of the cell and this was the impetus for a change in metabolic processes. It must be considered also that in our tests we did not use a clonal culture of P. pestis EV. in connection with which the possibility is not ruled out of the selective action of the sera used. Therefore, paramount importance belongs to carrying out analogous tests with pure lines of EV and other strains of the plague microbe.

The described phenomenon is also of interest in an epizootological aspect. It must be admitted that the immunological interrelationships of plague and certain enteric bacteria, encountered in rodents in regions which are enzootic for plague, are one of the factors for the mutability of P. pestis in nature. Thus, when mice, preliminarily immunized with E. coli communis No. 479, were infected with a virulent strain of the plague microbe, we isolated cultures, similar in their properties with the above described variants.

It is known that for the cultivation of the plague microbe extensive use is made of media containing the whole blood or serum of laboratory animals. Apparently when certain operations are being carried out it is necessary to conduct an investigation of the animal-donors from the viewpoint of exposing antibodies, active in respect to the plague microbe.

Conclusions

1. Mutability of the plague microbe (EV strain) was established as a result of the influence on it of sera against enteric bacteria.
2. A characteristic for the plague microbe, subjected to the influence of the stated sera, was the acquiring of features, inherent to the causative agent of rodent pseudotuberculosis.
3. The observed mutation of the plague microbe was not accompanied by changes in the DNA nucleotide composition.

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Table 1

Properties of certain variants of *P. pestis* EV, acquired under the influence of sera against enterobacteria.

Number of variant	Antiserum	Number of passage	Morphology of colonies	Growth on		Fermentation activity on media with				Relation to phages		Agglutination reaction with antiplague sera in dilutions of
				Acid-hungry agar	Peptone-free agar	Rhamnose	Glycerine	Urea	in reactions of	Plague	Pseudotuberculosis	
11	No 256	4	R	-	10 ⁻¹² *	+	+	+	nitritification	+	-	1:40
26	No 256	7	S	+	10 ⁻¹²	+	+	+	+	+	+	1:320
30	No 479	6	R	-	10 ⁻⁸	+	+	+	+	+	+	---
45	O 111	7	R	+	10 ⁻¹²	+	+	+	+	+	+	1:160
56	No 479	8	S	+	10 ⁻¹²	+	+	+	+	+	+	1:80
16	No 479	4	S	+	10 ⁻¹²	+	-	+	+	+	+	1:80
23	No 479	6	OR-OS	+	10 ⁻¹²	+	+	+	+	-	+	---
Initial		-	R	-	10 ⁻²	-	-	-	+	+	+	1:1 280

* The degree of dilution of a culture, the growth of which was accompanied by the growth of microbes: + positive result; - negative result.

Table 2

Nucleotide composition of DNA in variants of *P. pestis* EV, obtained under the influence of sera against enterobacteria.

Number of variant	Ratio of bases (in mole %)				Molar ratio		
	guanine	adenine	cytosine	thymine	$\frac{\text{Pur}}{\text{Pyr}}$	$\frac{\text{6-keto}}{\text{6-amino}}$	$\frac{\text{A + T}}{\text{G + C}}$
11	23.7 ± 0.06	25.5 ± 0.06	25 ± 0.09	25.8	0.97	0.98	1.05
26	24.3 ± 0.08	25.1 ± 0.08	24.9 ± 0.07	25.7 ± 0.07	0.97	1	1.03
30	24.3 ± 0.1	25.4 ± 0.07	24.7 ± 0.06	25.7 ± 0.1	0.98	1	1.04
45	24.3 ± 0.07	25.3 ± 0.07	24.8 ± 0.07	25.7 ± 0.08	0.98	0.99	1.04
56	24.1 ± 0.07	25 ± 0.06	24.7 ± 0.06	26.3 ± 0.08	0.96	1.01	1.05
16	24.1 ± 0.11	25.3 ± 0.07	24.8 ± 0.09	25.8 ± 0.07	0.93	1	1.05
23	24.2 ± 0.13	25.4 ± 0.06	24.8 ± 0.06	25.6 ± 0.12	0.98	0.99	1.04
Initial strain	23.4	26	25.4	25.2	0.94	0.95	1.05

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Legend: $\frac{\text{Pur}}{\text{Pyr}}$ - ratio of the total of purines (guanine + adenine) to the total of pyrimidines (cytosine + thymine); $\frac{\text{6-keto}}{\text{6-amino}}$ - ratio of the total of guanine and thymine to the total of adenine and cytosine; $\frac{\text{A + T}}{\text{G + C}}$ - ratio of the total of adenine and thymine to the total of guanine and cytosine.